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RESEARCH ARTICLE

# Nano seed priming with biogenic ZnO: Assessing the germination potential of sweet corn seeds

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## Abstract

Sweet corn (*Zea mays* var. *saccharata*) is a widely cultivated crop valued for its high sugar content and nutritional profile. Seed priming is a pre-sowing technique that enhances germination and seedling vigour and nanopriming has gained prominence with the advent of nanotechnology (NPs). This study aimed to standardize the green synthesis protocol for zinc oxide nanoparticles (ZnO NPs) using *Moringa oleifera* (MO) leaf extract and assess their impact on sweet corn seed priming. ZnO NPs were synthesized by co-precipitation method, characterized via XRD, FTIR, UV-Vis, TEM and EDAX analyses and subsequently applied in seed priming treatments at concentrations ranging from 100–500 ppm. The laboratory experiment, conducted in a Completely Randomized Design (CRD), evaluated germination parameters such as Germination Percentage (GP), Germination Rate (GR), Seedling Vigour Index (SVI), Mean Germination Time (MGT) and Coefficient of Velocity of Germination (CVG). Results revealed that ZnO NP priming significantly improved germination performance, with the 100 ppm as an ideal priming treatment exhibiting improvement in germination percentage (89.33 %), seedling length and vigour index (3873). The study suggests the potential of biogenically synthesized ZnO NPs in enhancing seed germination and seedling vigour, demonstrating their applicability as an eco-friendly priming agent These findings not only demonstrate the potential of green-synthesized ZnO NPs as an eco-friendly priming agent but also underscore their broader applicability in advancing sustainable agriculture through enhanced crop establishment and resource-efficient seed treatments.

**Keywords:** biogenic synthesis; seed priming; sweet corn; zinc oxide nanoparticle

## Introduction

Sweet corn (*Zea mays* var. *saccharata*) is globally known for its sweetness, flavor and nutritional content. Unlike field corn, it is harvested at the immature stage, making it a staple food and an essential raw material in the food industry. This hybrid variety, bred for higher sugar content, matures within 75-90 days after sowing. It contains approximately 5-6 % sugar, 10-11 % starch and 70 % water, along with moderate amounts of protein, vitamins and potassium (1). The choice of sweet corn variety varies regionally, with the standard yellow type being the most widely consumed.

Seed priming is a pre-sowing technique involving controlled hydration, enabling metabolic processes associated with germination to initiate without triggering radical emergence (2). During phase II of germination, priming facilitates seed hydration, activating pre-germinative biochemical and metabolic pathways while preventing radical protrusion (3, 4). This pre-germination improvement method promotes early seedling emergence by modulating metabolic activities during the initial stages of germination (5). Additionally, seed priming enhances germination rate and uniformity by shortening the imbibition phase (6), stimulating pre-germinative enzyme activity, boosting metabolite synthesis (7) and modulating osmotic balance. Various seed priming techniques, including water-based, Plant Growth Regulator (PGR)-based, osmotic solution-based and chemical-based methods, are extensively utilized to improve germination, seedling vigour and resistance to multiple biotic and abiotic stresses in numerous crop species. While all these approaches share a common principle partial pre-hydration and early activation of germination processes their effectiveness is largely influenced by the specific plant species and the selected priming method (8). Among these, the use of nanoparticles as priming agents has gained significant attention due to their unique physicochemical properties. Efficient priming agents, including $SiO_2$, Ag and ZnO, can be synthesized into NPs form and applied in seed priming

treatments to enhance seed germination and vigour (9, 10). A notable increase in shoot length and seed fresh weight was observed when treated with ZnO NPs at a concentration of 20 mg/L, compared to both the control and other priming methods (10).

The biogenic synthesis of NPs using plant extracts has emerged as a transformative approach, offering a distinct advantage over conventional methods due to its environmentally friendly nature and reduced ecological impact (11). Several studies have explored the green synthesis of ZnO NPs using various plant extracts. The physical properties and formation mechanisms of ZnO NPs synthesized using *Moringa oleifera* extracts (12). The structural and optical characteristics of ZnO NPs derived from *Aspalathus linearis* extracts (13). The fabrication of nanoscale electrocatalytic and optically modulated ZnO NPs via a green synthesis approach using *Punica granatum* L., along with their antibacterial properties (14). Additionally, the green synthesis of ZnO NPs utilizing *Agathosma betulina* extracts (15). In this study, we have used MO leaf extracts at 10 % concentration and 0.1 M zinc oxide hexahydrate following the co-precipitation method, optimised with unique and novel combinations of synthesis parameters, to obtain zinc oxide NPs for application in agriculture.

## Materials and Methods

The materials, methods and specific details of experiments conducted are described below.

### Nanoparticle synthesis and characterization

Zinc oxide NPs were synthesized using co-precipitation method (16). Biogenic synthesis of zinc oxide NPs was carried out using *Moringa oleifera* (MO) leaf extract as a reducing and stabilizing agent and zinc nitrate hexahydrate as precursor. MO leaves (15 g) were boiled in 150 ml of double distilled water (DD.$H_2O$), the solution was kept stirring at 850 rpm for 2 hrs at 50 °C. MO extract was cooled to room temperature and filtered through Whatman No.42 filter paper and stored in refrigerator at 4 °C for further studies. 50 mL of 0.1M Zinc nitrate hexahydrate solution was prepared with DD.$H_2O$. MO leaf extract (50 mL) was taken in a burette and added gently into 50 mL of zinc nitrate solution under constant stirring. pH was maintained at neutral by adding required volume of 2M Sodium hydroxide solution (NaOH). A yellow colour precipitate was obtained. Resultant solution was kept under vigorous stirring (500 rpm) at 90 °C for 4 hrs. After the solution was kept idle for 24 hrs in a sealed beaker for the formation of nanoparticle. Mixture was centrifuged at 3500 rpm for 30 min and the supernatant was discarded. The precipitate was washed with ethanol followed by distilled water and dried in hot air oven at 60 °C for 6 hrs. After fully drying, the particles were finely ground using a mortar and pestle, weighed and stored for future use. ZnO nano particles were annealed at 600 ° C for 2 hrs in muffle furnace. White powder of zinc oxide NPs was obtained and stored. The characterization of the synthesized NPs was carried out using TEM with EDAX, XRD, FTIR and UV-Vis Spectroscopy.

### The seed priming experiment

The laboratory study was conducted at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore to evaluate the impact of seed priming, using the synthesized nanoparticles, on germination and growth performance of sweet corn variety 'Sugar 75'. The experiment was laid out using CRD with seven treatments replicated thrice The duration for seed priming was standardized with a preliminary experiment in which three replicates of 25 seeds each were primed for 2, 4, 6, 8 and 10 hrs and then kept for germination test by roll towel method in a controlled germination room maintained under standard conditions of 25 ± 2 °C temperature and 95 ± 2 % relative humidity. The best duration was identified to be 4 hrs by assessing germination percentage, seedling length and vigour index.

The priming was carried out for 4 hrs in 250 mL glass beakers, with a concentration of ZnO NPs prepared by green synthesis methods following the detailed protocol described previously and later kept for germination. The seed priming treatments given were $T_1$- 100 ppm, $T_2$-200 ppm, $T_3$ - 300 ppm, $T_4$ - 400 ppm, $T_5$ - 500 ppm, $T_6$ - Priming with distilled water and $T_7$ - No priming. After priming, seeds were air-dried and kept for germination test by roll towel method (three replicates, each of 25 seeds) and top of paper method (three replicates each of 10 seeds), in the germination chamber under standard conditions as mentioned above. The growth performance of the primed seeds was observed after 7 days, while germination count was observed daily. The parameters like daily germination count, shoot length and root length were recorded. Germination Percentage (GP), Seedling Vigour Index (SVI), Germination Rate (GR), Mean Germination Time (MGT) and Co-efficient of Velocity of Germination (CVG), were computed later with the observed data. The fresh weight of seedlings was recorded and the samples were subjected to drying at 72 °C in a hot air oven for recording the seedling dry weight. The GP, GR, MGT and CVG were calculated using the following formula given by (17):

GP (%) = (No. of normal seedlings/Total No. of seeds sown) x 100 (Eqn. 1)

$$GR = \Sigma (n_i / d_i) \quad \text{(Eqn. 2)}$$

$$MGT = \Sigma (n_i \times d_i) / \Sigma n_i \quad \text{(Eqn. 3)}$$

$$CVG = \Sigma n_i / \Sigma (d_i \times n_i) \quad \text{(Eqn. 4)}$$

$n_i$ - number of seeds germinated on $i^{th}$ day

$d_i$ -number of days counted from the beginning of experiment

SVI was calculated using the formula given by (18) and expressed in whole number,

SVI = GP (%) x Seedling length (cm) {Shoot length + Root length} (Eqn. 5)

### Statistical analysis

Analysis of variance (ANOVA) technique was used to analyse the significance of different treatments and the LSD test at $P \leq 0.05$ was used to compare treatment means by using GRAPES (General R based Analysis Platform Empowered by Statistics) computer-based software (19). Correlation analysis was done using R software.

## Results and Discussion

### Nanoparticle synthesis

In this study, we have used the co-precipitation method for synthesis of zinc oxide NPs as it is one of the easy and cost-effective methods for synthesis of metal oxide nanoparticles (20-22). Various researchers have used different chemical combinations of precursors and reducing agents for synthesis of ZnO NPs. (12) obtained ZnO NPs of particle size in the range of 12-20 nm, with MO leaf extract as biological reducing agent. (13) synthesized ZnO NPs with size of 12 nm using *Aspalathus linearis* extract as reducing agent. Similarly, (15) and (14), used *Agasthoma betulina* and *Punica granatum* respectively, as reducing agents in the production of ZnO NPs of size 19-20 nm. We have used zinc nitrate hexahydrate as precursor in the co-precipitation method and obtained nanoparticles of average particle size of 24 nm based on TEM result. For this size achievement, MO leaf extract worked as the reducing and stabilizing agent as MO is reported to be composed of several biomolecules like flavonoids, saponins, tannins, (23) phenolics, crypto-chlorogenic acid, isoquercetin, astragalin (24) and vitamins like L-ascorbic acid and niacin (25) which may have contributed for the size reduction.

### Zinc oxide nanoparticle characterization

#### 1. XRD

The X-ray diffraction (XRD) analysis of the synthesized ZnO NPs, using *Moringa oleifera* leaf extract, revealed the presence of a crystalline phase in the NPs. The XRD pattern (Fig. 1) displays prominent peaks corresponding to various crystallographic planes. Notably, distinct diffraction peaks were observed at 2θ values of 31.7°, 34.4°, 36.2°, 47.5°, 56.6° and 62.8°. These peaks are indexed to the (100), (002), (101), (102), (110) and (103) planes of hexagonal wurtzite ZnO, confirming the formation of pure ZnO NPs (JCPDS card no. 36 -1451). The sharpness and intensity of these peaks suggest the high crystallinity of the synthesized NPs when compared to (26) and (27).

#### 2. FTIR

The FTIR spectrum provided further insight into the functional groups involved in the biosynthesis of ZnO nanoparticles (Fig. 2). A broad absorption band between 2978 $cm^{-1}$ and 2900 $cm^{-1}$ was attributed to O-H stretching vibrations, indicating the presence of hydroxyl groups from the *Moringa* extract. Peaks observed at 1381.03 $cm^{-1}$ were attributed to C-H bending (methyl groups) suggesting the interaction of carboxylate groups with the ZnO surface. Additional bands in the 1149.57-1249.87 $cm^{-1}$ region correspond to C-O-C stretching vibrations, suggesting the presence of esters or ethers, likely derived from polysaccharides or other biomolecules in the extract. Strong Zn-O stretching vibrations were identified at 455.20 $cm^{-1}$ and 432.05 $cm^{-1}$, confirming the formation of ZnO nanoparticles. The presence of these functional groups highlights the role of *Moringa oleifera* extract in reducing and capping the ZnO nanoparticles, ensuring their stability (28). These results demonstrate the efficacy of using *Moringa oleifera* leaf extract as a green synthesis method for ZnO nanoparticles, providing a sustainable and environmentally friendly alternative to conventional chemical synthesis techniques.

#### 3. UV–Vis spectra

The UV-Vis absorption spectrum of ZnO (Fig. 3) NPs synthesized using *MO* leaf extract displays a prominent absorption peak at 330 nm. This peak corresponds to the characteristic excitonic absorption of ZnO NPs, indicating the successful formation of nanosized particles. The absorption in this region confirms the presence of ZnO, as it is consistent with the band gap of ZnO NPs, typically ranging from 3.3 eV, corresponding to a wavelength of around 370 nm (29). The

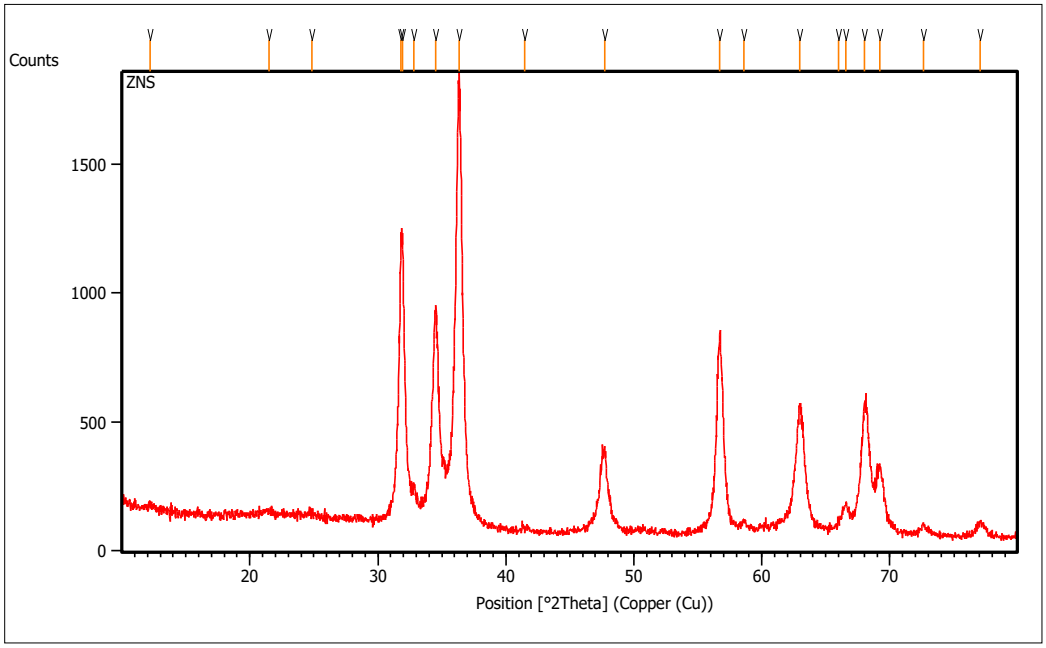


**Fig. 1.** XRD spectra of the green synthesized ZnO NPs.

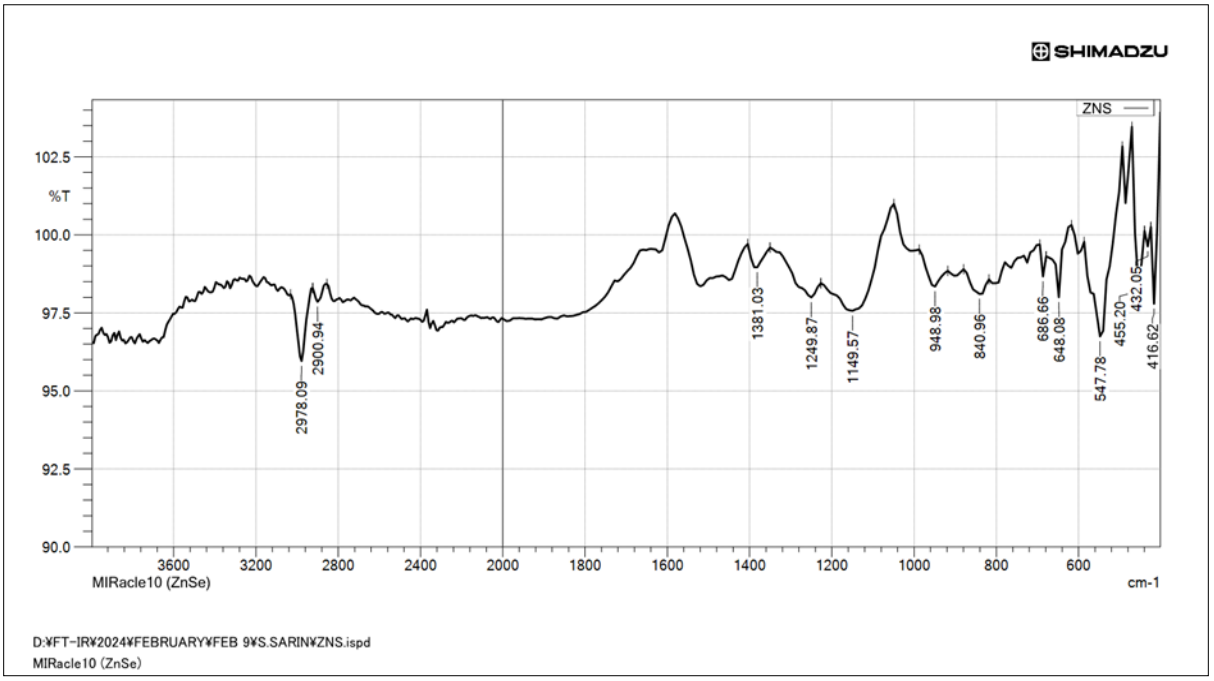


**Fig. 2.** FTIR spectra.

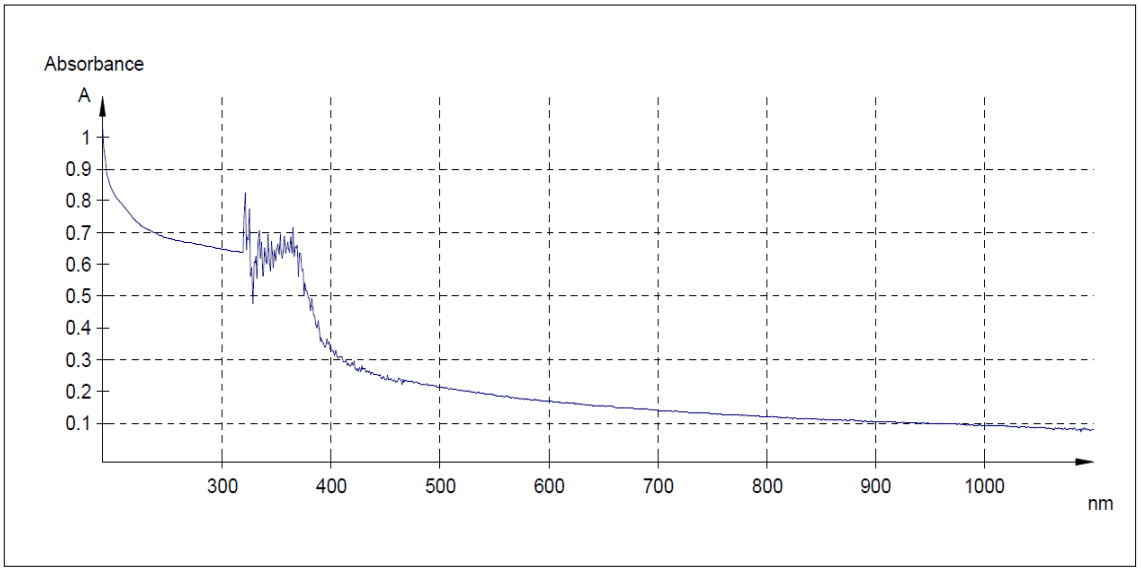


**Fig. 3.** UV-Vis.

observed blue shift in the absorption spectrum, compared to bulk ZnO, can be attributed to the quantum confinement effect. This shift is indicative of a reduction in particle size, leading to an increase in the band gap energy. The slight fluctuations in the absorbance before and after 330 nm may result from slight variations in particle size distribution or surface defects.

**4. TEM analysis**

Zinc oxide NPs were characterized by Transmission Electron Microscopy (TEM) to evaluate particle size, morphology and agglomeration. The TEM image (Fig. 4) reveals a heterogeneous distribution of ZnO NPs with sizes ranging from 16 nm to 51 nm. The NPs exhibit an average size distribution predominantly within the 20-30 nm range.

The morphology of the ZnO NPs appears predominantly quasi-spherical, in nature. These agglomerates could be indicative of weak van der Waals interactions or the influence of biomolecules acting as capping agents during synthesis. The variation in particle size may result from the natural complity of green synthesis methods, where factors such as extract concentration, temperature and reaction time influence NPs growth. Despite these variations, the ZnO NPs synthesized via this eco-friendly, plant-mediated method exhibit well-defined nanoscale properties, supporting the efficacy of *Moringa oleifera* extract as a reducing and stabilizing agent (12).

**5. EDAX**

X-ray (EDX) techniques were employed to further investigate and obtain more information about the elemental composition of the ZnO NPs. The spectra displayed in Fig. 5 reveal three distinct zinc peaks at energies of 1.01 keV, 8.63 keV and 9.57 keV, alongside a single oxygen peak at 0.53 keV, all associated with ZnO NPs. The high intensities of the zinc and oxygen peaks indicate that the predominant component of the sample is ZnO. The two peaks of copper lying between 8.04 keV and 10keV indicate the presence of copper, which is present in the grid used in sample coating during TEM imaging.

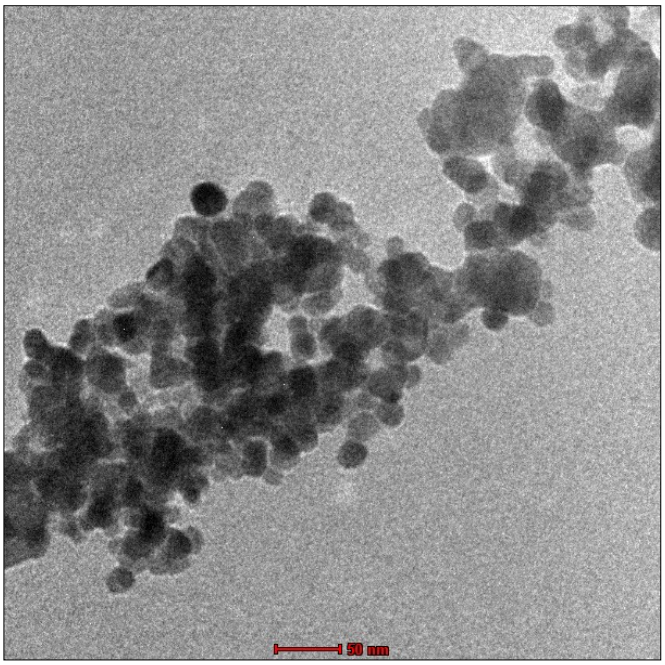


**Fig. 4.** TEM Image.

## Germination and growth parameters

Seed priming using green synthesized ZnO-NPs had significant effect on various germination parameters in sweet corn (Table 1).

### 1. Germination percentage

The highest germination percentage was observed in $T_1$ (89.33 %), which was significantly higher compared to $T_7$ (62.67 %), the control. This result is consistent with studies indicating that priming with ZnO NPs can enhance seed germination due to improved water uptake and cellular activities during germination (30, 31). Treatment $T_2$ (84 %) also exhibited high germination percentages, suggesting a dose-dependent effect of ZnO NPs concentration.

### 2. Shoot and root length

Seed priming with ZnO NPs at 100 ppm ($T_1$) resulted in the highest shoot (19.14 cm) and root (24.20 cm) lengths. In contrast, $T_7$ (control) showed the lowest shoot and root lengths, emphasizing the positive impact of priming treatments. Zinc is involved in the biosynthesis of endogenous hormones like auxins and gibberellins, which are crucial for cell division and elongation, thus supporting efficient growth and development of seedlings (32). Similar results were noted in maize (31). ZnO NPs at lower concentrations enhanced seedling growth in rice, higher concentrations had negative effects on early growth stages. The study suggested that excessive ZnO NPs could induce oxidative stress, leading to reduced root and shoot lengths, which explains the reduction in growth and germination in higher doses (33).

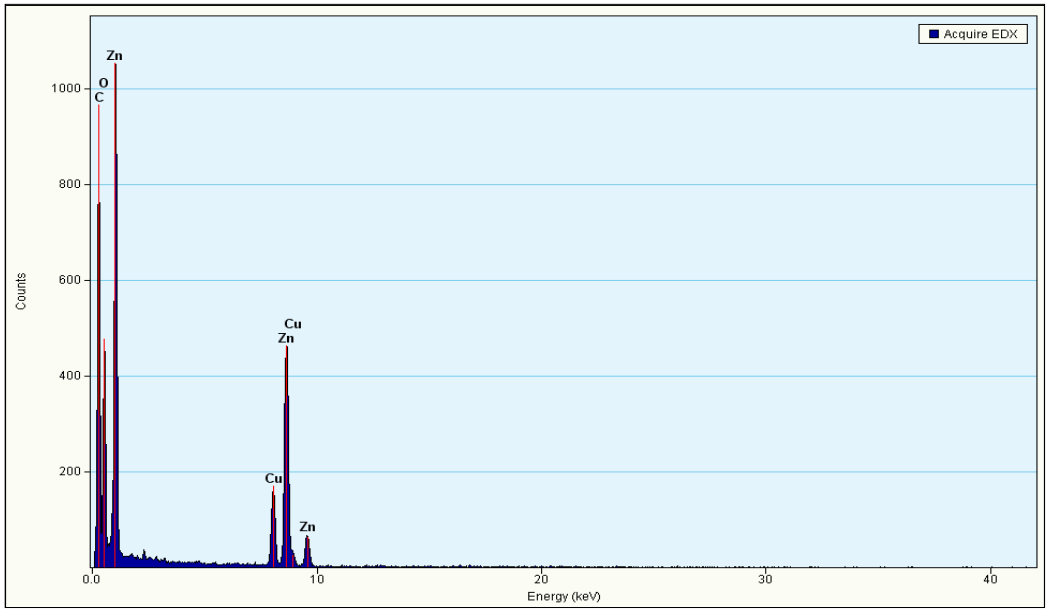


**Fig. 5**. EDAX.

**Table 1.** Effect of seed priming with zinc oxide nanoparticles on germination parameters in roll towel method

| Treatments | GP (%) | SFW (g) | SDW (g) | SL (cm) | RL (cm) | SVI |
|---|---|---|---|---|---|---|
| T1 | 1.11 (89.33) | 4.94 | 0.52 | 19.14 | 24.20 | 3873 |
| T2 | 1.01 (84.00) | 4.82 | 0.49 | 16.84 | 23.20 | 3357 |
| T3 | 0.88 (76.67) | 4.67 | 0.45 | 16.54 | 22.40 | 2982 |
| T4 | 0.79 (70.67) | 4.30 | 0.44 | 14.30 | 21.30 | 2523 |
| T5 | 0.75 (68.00) | 4.20 | 0.40 | 13.60 | 21.10 | 2367 |
| T6 | 0.80 (72.00) | 4.13 | 0.38 | 13.03 | 20.70 | 2428 |
| T7 | 0.68 (62.67) | 3.88 | 0.35 | 11.10 | 19.50 | 1916 |
| **SE(d)** | **0.06** | **0.10** | **0.03** | **1.01** | **1.04** | |
| **LSD** | **0.133** | **0.209** | **0.058** | **2.156** | **2.236** | |
| **p-value** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.008** | |

(GP - Germination Percentage; SFW - Seedling Fresh Weight; SDW - Seedling Dry Weight; SL - Shoot Length; RL - Root Length; SVI - Seedling Vigour Index *Germination percentage data was arc sine transformed

### 3. Seedling Vigour Index (SVI)

The highest SVI was observed in $T_1$ (3873), which was significantly higher than the control (1916). The SVI, a reliable indicator of seedling vigour, reflects the overall quality of seedlings. Similar results in wheat, which indicate that ZnO NP priming at lower concentrations can significantly improve seedling vigour, likely due to the enhanced early growth and root development (34). ZnO NPs regulate amylase activity, accelerating the hydrolysis of starch into soluble sugars, which provide essential energy for radicle emergence and seedling establishment, thereby enhancing SVI. Additionally, zinc stabilizes plasma membranes by modulating membrane-bound phospholipids and proteins, leading to improved water uptake efficiency. This facilitates higher osmotic adjustment, resulting in greater seed turgidity and uniform germination (35), which may have contributed to the superior SVI observed in $T_1$.

### 4. Seedling fresh and dry weight

$T_1$ (100 ppm) also recorded the highest fresh (4.94 g) and dry (0.52 g) weights, indicating that ZnO NPs priming not only enhances seedling growth but also contributes to better biomass accumulation. The findings of this study are in line with reports that NPs priming improves metabolic processes that contribute to increased biomass production (30). Zinc is a cofactor for numerous enzymes involved in protein metabolism and carbohydrate development and such enzymatic activity being vital for energy production, may have led to improved biomass accumulation in growing seedlings (36).

### 5. GR, MGT and CVG

The data pertaining to GR, MGT and CVG is shown in Table 2. The highest germination rate (5.75) and the highest CVG (0.53) were observed in $T_1$, indicating that ZnO NPs priming accelerates germination speed, which is essential for uniform crop establishment. Conversely, the highest MGT (2.52) was observed in $T_7$ (control), suggesting slower germination in untreated seeds. A reduced MGT and increased CVG reflect improved seedling establishment and early growth, as observed by (37). The highest coefficient of velocity of germination (CVG) was observed in seeds treated with ZnO NPs suspensions at 150 mg/L, showing a 14.7 % increase compared to the control. Furthermore, ZnO NP treatments at 50 mg/L and 250 mg/L resulted in CVG improvements of 9.1 % and 3.5 %, respectively, over the control (38). Similarly, ZnO NPs at 100 mg/L significantly accelerated seed germination in pea cultivars, increasing germination rates from 60 % to 80 %, while higher concentrations did not produce notable effects on germination speed (39).

### Correlation analysis

The correlation analysis (Fig. 6) revealed strong positive associations between germination percentage (GP) and key seedling traits, including shoot length (r = 0.95), root length (r = 0.97), seedling vigour index (r = 0.97) and fresh weight (r = 0.95). This suggests that effective priming treatments, particularly ZnO NPs, enhance both germination success and early seedling growth by improving water uptake, enzyme activation and metabolic processes. A near-perfect correlation (r = 0.99) between shoot and root length suggests that improved shoot growth may be associated with a proportional root elongation,

**Table 2.** Effect of seed priming with zinc oxide nanoparticles on germination parameters in top of paper method

| Treatments | GR | MGT | CVG |
|---|---|---|---|
| T1 | 5.75 | 1.89 | 0.53 |
| T2 | 4.97 | 2.10 | 0.47 |
| T3 | 4.84 | 2.18 | 0.46 |
| T4 | 4.54 | 2.19 | 0.46 |
| T5 | 4.09 | 2.21 | 0.45 |
| T6 | 3.55 | 2.29 | 0.44 |
| T7 | 3.37 | 2.52 | 0.40 |
| **SE(d)** | **0.11** | **0.01** | **0.02** |
| **LSD** | **0.227** | **0.213** | **0.044** |
| **p-value** | **<0.001** | **0.001** | **0.001** |

(GR - Germination Rate; MGT - Mean Germination Time; CVG - Coefficient of Velocity of Germination)

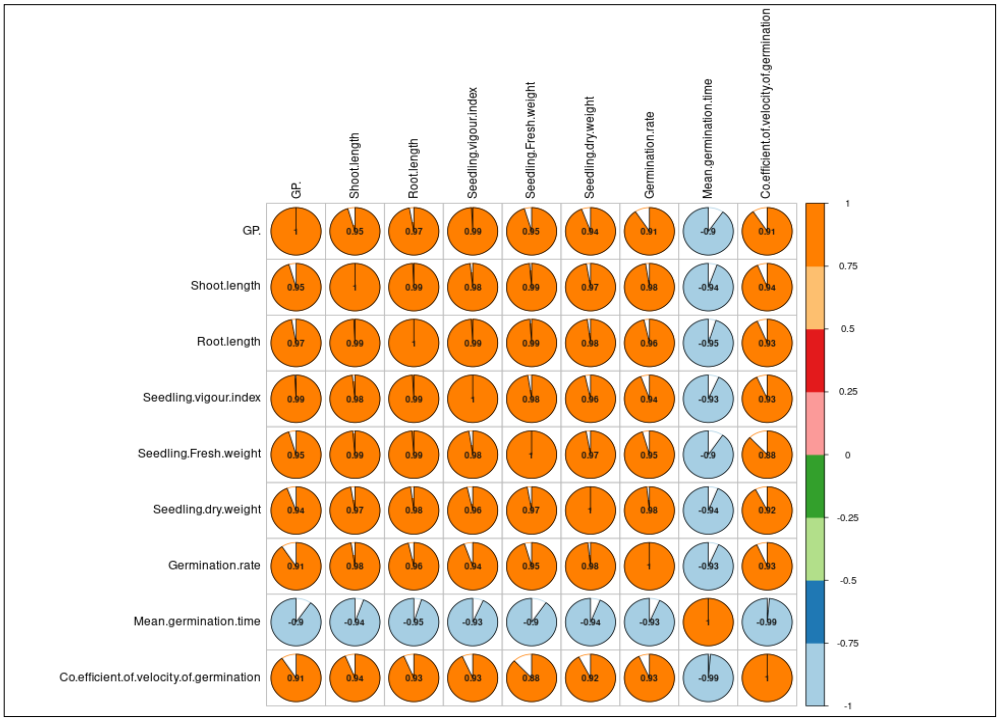


**Fig. 6.** Correlation plot showing relationship between different germination parameters.

which in turn is linked to enhanced seedling vigour. However, it is important to note that this correlation does not confirm a direct causal relationship, as both growth parameters are influenced by a range of physiological and environmental factors. Additionally, the strong association of seedling dry weight with fresh weight (r = 0.97) suggests efficient biomass accumulation in well-primed seeds, likely due to enhanced nutrient uptake and metabolic activity.

Germination kinetics showed an inverse relationship between mean germination time (MGT) and growth parameters, with MGT negatively correlated with GP (r = -0.90), shoot length (r = -0.94), root length (r = -0.95) and seedling vigour index (r = -0.93). This indicates that treatments promoting faster germination also resulted in better seedling development. The CVG exhibited strong positive correlations with GP (r = 0.91), shoot length (r = 0.94) and germination rate (r = 0.93), reinforcing the role of ZnO priming in synchronizing germination. These findings suggest that ZnO NPs enhance seedling establishment by accelerating germination and improving early growth, though further studies are needed to optimize concentrations and mitigate potential toxicity at higher doses.

## Conclusion

The ZnO-NPs synthesized using *Moringa oleifera leaf extract* as a reducing and stabilizing agent, yielded spherical nanoparticles with an average particle size of 24 nm was confirmed using TEM and X-ray diffraction (XRD). Seed priming sweet corn seeds with ZnO nanoparticles, at 100 ppm, significantly enhances germination parameters. This study provides evidence supporting the potential of ZnO nanoparticle priming as an effective strategy to improve seed performance and seedling vigour in sweet corn, offering a sustainable solution for enhancing crop production. At higher concentrations, ZnO NPs may induce mild oxidative stress, which may negatively affect the growth and germination. As a future line of work, one can consider conducting field trials using green synthesized ZnO NPs for seed priming in different sweet corn varieties to confirm laboratory findings under real -world conditions. Further refine the optimal ZnO NP concentration by testing a narrower range to pinpoint the most effective dosage for field conditions. Also assess the performance of ZnO NP-primed sweet corn under mild abiotic stress conditions (e.g., slight drought or salinity) to determine if priming offers additional benefits in stress mitigation.

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## Authors' contributions

$SS^1$ carried out the experiment, recording of observations, analysis of the data and preparation of the manuscript. $SS^2$ guided the research by formulating the research concept and reviewed and approved the final manuscript. VM guided the research by formulating the seed priming research concept and facilitated analysis. SKR guided research in nanoparticle synthesis, characterization, application and helped in editing, summarizing and revising the manuscript. PSM shared their inputs for upscaling and guided the research in characterization, bulking nanoparticle and its application. Also helped in editing summarizing and revising the manuscript. SM helped in formulating the nanoparticle research concept and revising the manuscript. GG shared their inputs and helped in interpretation of characterization analysis, summarizing, editing and revising the manuscript. ($SS^1$stands for S Sarin and $SS^2$ stands for S Sanbagavalli).

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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